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Baseline Study of Effects of Ionizing Radiation on the Chromosomes of the Marine Worm 'Neanthes arenaceodentata'

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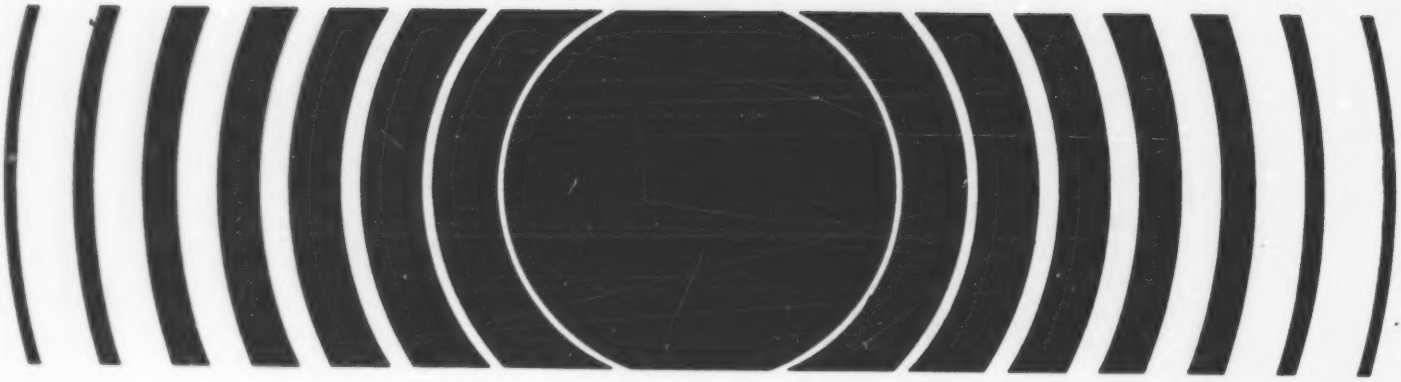

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16. ABSTRACT

Between 1946 and 1970 the United States disposed of low level radioactive waste at several sites in the Atlantic and Pacific ocean. In 1972 the Environmental Protection Agency was authorized under the Marine Protection Research and Sanctuaries Act (PL-92-532) to regulate all ocean disposal activities. This Act requires EPA to establish a program for reviewing and evaluating ocean dumping permit applications. Before EPA can approve permits for ocean dumping of radioactive waste, it will be necessary to determine how such wastes may affect marine biota. The purpose of this study was to test the feasibility of using a marine coastal worm, Neanthes arenaceodentata, as a cytogenetic model for assessing radiation damage to ocean organisms. Groups of worms were exposed to cobalt-60, then slide preparations were made and scored for gross chromosome aberrations and damage. All the levels of ionizing radiation tested from180-680 rads were found to cause significant chromosome damage in N. arenaceodentata. The nature of this damage depended on dose, dose-rate and DNA repair capability of the worm. Worms responded to the same dose range as do mice. Since mice are accepted models for studying radiation effects on humans, this study supports the utility of using this species to study radiation effects on marine organisms.

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Baseline Study of Effects of Ionizing Radiation on the Chromosomes of the Marine Worm, Neanthes Arenaceodentata*

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FOREWORD

The Environmental Protection Agency, (EPA), was given a Congressional mandate to develop criteria, standards, and regulations governing the ocean disposal of all forms of wastes pursuant to Public Law 92-532, the Marine Protection, Research and Sanctuaries Act of 1972. Within this Congressional mandate, EPA has initiated a specific program to develop regulations and criteria to control the ocean disposal of radioactive wastes.

EPA has taken an active role both domestically and within the international nuclear regulatory arena to develop the effective controls necessary to protect the health and safety of man and the marine environment. The EPA Office of Radiation Programs (ORP) initiated feasibility studies to determine whether current technologies could be applied toward determining the fate of radioactive wastes dumped in the past. After successfully locating actual radioactive waste containers in three of the primary radioactive waste disposal sites used by the United States in the past, ORP developed an intensive program of dumpsite characterization studies to investigate the following: (a) the biological, chemical and physical parameters, (b) the presence and distribution of radionuclides within these sites, and (c) the performance of past packaging techniques and materials.

These studies have provided needed information and data on the past and present nuclear waste disposal activities concommitant with the growing national and international concern for the long-term effects of this low-level waste disposal option.

This paper describes a preliminary investigation of the utility of a cytogenetics technique for detecting potential biological effects which might arise from the practice of low-level radioactive waste disposal at sea. This initial effort was undertaken to determine whether a marine polychaete, Neanthes, would be a suitable test organism for a cytogenetics study of effects of external radiation. It is intended that future studies use lower levels of radiation, internal as well as external, in order to better simulate those exposure levels and modes likely to occur in deep-sea low-level radioactive waste disposal sites.

Abstract

Between 1946 and 1970 the United States disposed of low level radioactive waste at several sites primarily in the Atlantic and Pacific ocean. In 1972 the Environmental Protection Agency was authorized under the Marine Protection Research and Sanctuaries Act (PL-92-532) to regulate all ocean disposal activities, including any future ocean disposal of radioactive material. This Act requires EPA to establish a program for reviewing and evaluating ocean dumping permit applications. EPA is now establishing a permit program. Before EPA can approve permits for ocean dumping of radioactive waste, it will be necessary to determine how such wastes may affect marine biota.

The purpose of this study was to test the feasibility of using a marine coastal worm, Neanthes arenaceodentata, as a cytogenetic model for assessing radiation damage to ocean organisms. Groups of worms were exposed to cobalt-60, 180-680 rads, at the Battelle Northwest Laboratory, then shipped to the EPA-Environmental Research Laboratory at Narragansett, R.I., where slide preparations were made and scored for gross chromosome aberrations and damage. All the levels of ionizing radiation tested from 180-680 rads were found to cause significant chromosome damage in N. arenaceodentata. The nature of this damage depended on dose, dose-rate and DNA repair capability of the worm. Worms responded to the same dose range as do mice. Since mice are accepted models for studying radiation effects on humans, this study supports the utility of using this species to study radiation effects on marine organisms. The radiation doses used in this study are considerably higher than the expected doses that would arise from the highest measured concentration of a radioactive isotope found in any U.S. deep-sea dumpsite

(cesium-137 in the Atlantic 2800 meter dumpsite from samples collected by EPA in 1976). Now that the feasibility of this test system has been shown, it is recommended that future study use lower doses.

Introduction

Low-level radioactive wastes, primarily from radionuclides used in hospitals, laboratories and industry were disposed of by the United States mainly at a site near the Farallon Islands in the Pacific Ocean from 1946 to 1966 and at two sites in the Atlantic Ocean from 1951 to 1967 (NAS, 1959; NAS, 1962; NAS, 1971). These wastes were usually packaged in ordinary 55 gallon steel drums with concrete covers or linings. The EPA-Office of Radiation Programs has investigated the Pacific site with subsite depths of 900 m and 1700 m and the two Atlantic sites at depths of 2800 m and 3800 m. At each site, waste drums were located with the use of a submersible vehicle, and low concentrations of radionuclides were found in the sediments and attributed to leaking or leaching from some of the containers (Dyer, 1976).

There has been limited research on the effects that might be expected for marine organisms exposed to low radioactivity concentrations measured during these site surveys. Before any future dumping of radioactive wastes can be permitted, it is important to learn if genetic effects may be caused by increased radionuclide concentrations in the marine environment, and whether or not these effects can be quantified.

In the deep sea, diversity is high, but each species population is in low abundance. It is believed that the distribution of individual species

in the deep sea is restricted and may, in some cases, even be limited to the area of a single basin (Hessler and Jumars, 1977). If this is so, then an influx of radioactive materials having the potential to cause genetic effects may significantly alter the gene pool of local populations.

Little is known of the genetic effects of man-produced radionuclides on marine species (NAS, 1971; Egami, 1980; Templeton, 1980). The International Atomic Energy Agency (1979) recommended that cytogenetics "may be the primary means of assessing the radiosensitivity of deep-sea fauna or, more likely, their shallow water counterparts." An *in vivo* cytogenetic model system using a coastal marine polychaete, *N. arenaceodentata*, has been recommended for marine genetic toxicology (Pesch and Pesch, 1980). This species has a suitable karyotype; is easily handled and cultured in the laboratory; is widely used in marine toxicological research; and sensitive cytogenetic procedures have been established (Pesch and Pesch, 1980). Deep-sea species of *Neanthes* have been found in the radioactive waste disposal sites under investigation (Reish, 1978). The purpose of this study is to test the feasibility of using this species as a test animal for studying radiation effects on marine organisms.

Materials and Methods

Juvenile *N. arenaceodentata* were obtained from Donald Reish, California State University, Long Beach, California. These worms were irradiated and fixed by James S. Young, PNL Marine Research Laboratory, Sequim, Washington. Groups of 40 worms each were placed in 1 liter polyethylene jars filled with filtered seawater. Each jar was aerated and the worms were fed an excess of dried *Enteromorpha* sp. twice weekly. The groups were subjected to either acute or chronic dose rates as follows: One group was

irradiated one minute with a Gamma Beam 650 panoramic irradiator (Atomic Energy Ltd., Canada) housed in a room 24 feet square with high-density concrete walls. The sample was placed centrally between 2 of 12 available source tubes. Each of the two source tubes contained approximately 1200 Ci of cobalt-60. The distance between the tubes was 80 cm and they were approximately 1.5 meters from the floor with the jars supported by polyethylene foam. The dose was 225 $\pm$ 13 rads (the $\pm$ value represents one standard deviation)) measured by placing five lithium fluoride thermoluminescent dosimeters (TLDs) under each jar. A control set of TLDs read 40 $\pm$ 1 millirad. A second group was irradiated at the same dose rate but for 3 minutes for a total dose of 680 $\pm$ 20 rads. Another group was exposed to a neutron activated stainless steel strip (0.51 x 8.9 x 25 mm) containing 0.854 mCi of cobalt-60 as the major radioisotope. The strip was activated in 1973, so most of the iron 55 and other short lived isotopes had decayed away. The median distance for the worms from the source was 7 cm. The dose was 180 $\pm$ 10 rads after 24 days exposure. Another group was exposed to a neutron activated stainless steel strip (0.51 x 8.9 x 25 mm) containing 1.18 mCi cobalt-60 for 48 days. The dose was 677 $\pm$ 153 rads. The radiation dosages of the latter two groups were measured by taping five TLDs to the side of each jar so they were 7 cm from the source. Control worms were treated similarly but received no irradiation.

The worms were prepared for shipment to ERL Narragansett by placing them overnight in 0.05% colchicine in filtered seawater with food, washing them in filtered seawater, placing them in 0.4% KCl in distilled water for 30 minutes, then fixing them in a chilled 3:1 mixture of absolute ethyl alcohol and glacial acetic acid, 3 changes, 15 minutes each. Storage was in the last change of fixative. Each group of worms was coded so that the cytogenetic observations were made without bias.

Immediately upon receipt at the Narragansett Environmental Research Laboratory, the fixative was renewed and the samples were refrigerated. Slides were prepared for 10 individuals from each group. The length of each worm was measured and its segments counted. Individual worms were then macerated and digested separately in 60% acetic acid for approximately one minute and all the material applied to clean slides, the number depending upon the size of each worm. Slides were prepared and stained with 4% Giesma for 5 minutes using the procedure of Kligerman and Bloom (1977). The entire worm represented on slides was screened.

For each group, either 10 worms or 300 metaphases were scored, whichever came first. For each worm scored, every metaphase on all slides for that worm was counted. Each metaphase was scored as normal, abnormal (one or more abnormal chromosomes), or as non-scorable (not spread well enough to make a judgment). Observations were made with a research microscope at 1250X, oil immersion. Photographs were taken at 1000X with a green filter using high contrast copy film. Differences in the data were tested for significance by a comparison of proportions test for binomial distributions (Snedecor and Cochran, 1967). An alpha of 0.05 was used unless otherwise noted.

Results

A total of 48 worms representing 4 different radiation treatments and 2 controls was counted. Each worm was evaluated individually. The quality of the chromosome preparations was poor due to the extended time in colchicine (17 hrs) and the transport in fixative. Those cells with chromosomes spread well enough in metaphase to observe (1250X, oil immersion) were scored as

either normal or abnormal. The abnormal designation meant that one or more chromosomes in the cell were distorted or broken. Representative examples may be seen in figure 1. All of the irradiated groups exhibited significant increases in abnormal metaphases (Table 1).

The low dose rate groups had significantly fewer abnormal metaphase cells than the high dose rate groups (Figure 2). Within the low dose rate group the low dose group had significantly more abnormal metaphase cells than the high dose group. The low dose rate groups had greater numbers of cells with single chromatid or chromosome damage while the high dose rate groups had greater numbers of cells with multiple chromosomes damaged, or granular or amorphous chromosomes (Table 1).

The number of metaphase cells per individual proved to be quite variable, ranging from 0 to 345. Even within a single control group, total metaphase counts ranged over 100 fold between individuals.

Discussion

Information is needed about genetic effects of radiation on marine organisms. It is difficult to collect this information in the field, particularly in deep-ocean environments. Instead, shallow water species, tested in controlled laboratory studies, may serve as surrogate models for measuring potential genetic effects on deep-ocean fauna exposed to radioactive materials. Ideally, we would be able to apply a variety of genetic tests using these model species, but our present lack of knowledge about the genetics of marine species does not permit this. The best approach at this time for studying genetic damage to marine organisms is proposed to be cytogenetic (IAEA, 1979; Kligerman, 1980). Cytogenetic techniques are reasonably simple and inexpensive.

Best estimates of aberration frequency are made by observing the first metaphase following their induction (Evans, 1976). This was done in the present study for high dose rates. Further, by arresting cells at metaphase (e.g., with colchicine), observations on chromosomes are possible in a wide variety of tissues. N. arenaceodentata represents a simple model with 18 relatively large chromosomes, for which metaphase techniques have been developed (Pesch and Pesch, 1980). The present study tests whether this species is a suitable model for studying radiation effects on marine organisms. The effects of radiation on mammalian chromosomes are well characterized; see for example, the review by Evans (1974). Significantly increased frequency of aberrations of mammalian, including human, chromosomes are commonly found at doses between 10 and 600 rads (Evans, 1974).

The doses used in the present study are considerably higher than those expected in contaminated aquatic environments. Natural background radiation in aquatic sediment is approximately 350 millirads/year (Templeton, 1976), while contaminated sediments such as the northeast Irish sea in the immediate vicinity of discharge from the Windscale reprocessing plant may experience levels above 200 rads/year (Templeton, 1976).

In summary, ionizing radiation at high doses does cause genetic damage in the marine worm, N. arenaceodentata. Clearly, there is a need to conduct studies which accurately simulate conditions at waste disposal sites. The doses administered in this study are considerably higher than those expected to occur from the highest concentrations of radionuclides measured in past-used ocean radioactive waste disposal sites, (cesium-137, Atlantic 2800 m dumpsite (Bowen, 1980)) but were used in order to establish cytogenetic technique capability. It is recommended that future studies use lower doses.

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Figure Legends

Fig. 1. Photographs of N. arenaceodentata chromosomes: A. normal control; B. chromatid gap, chronic high dose; C. broken chromatid, chronic high dose; D. broken chromosome, chronic high dose; E. granular chromosomes, acute low dose; F. amorphous chromosomes, acute high dose.

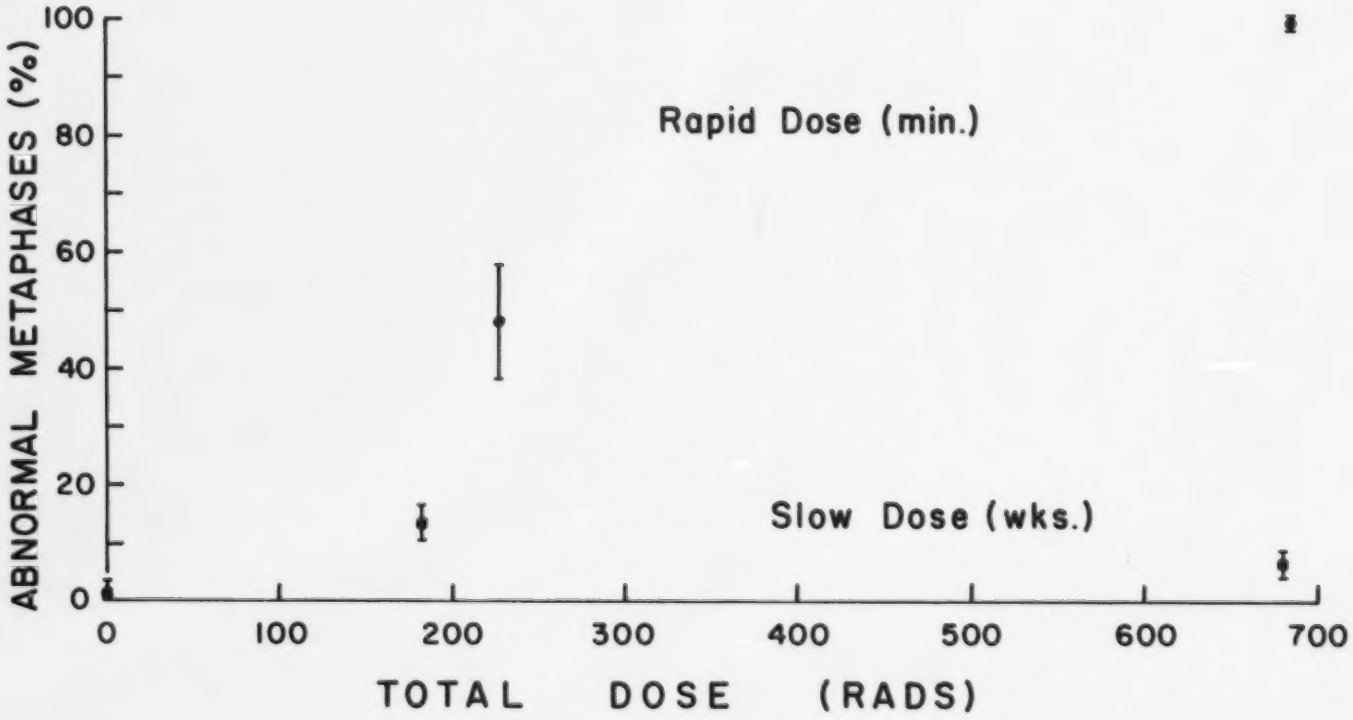
Fig. 2. Dose-response of N. arenaceodentata chromosomes to rapid and slow exposure of gamma radiation from a Cobalt-60 source.

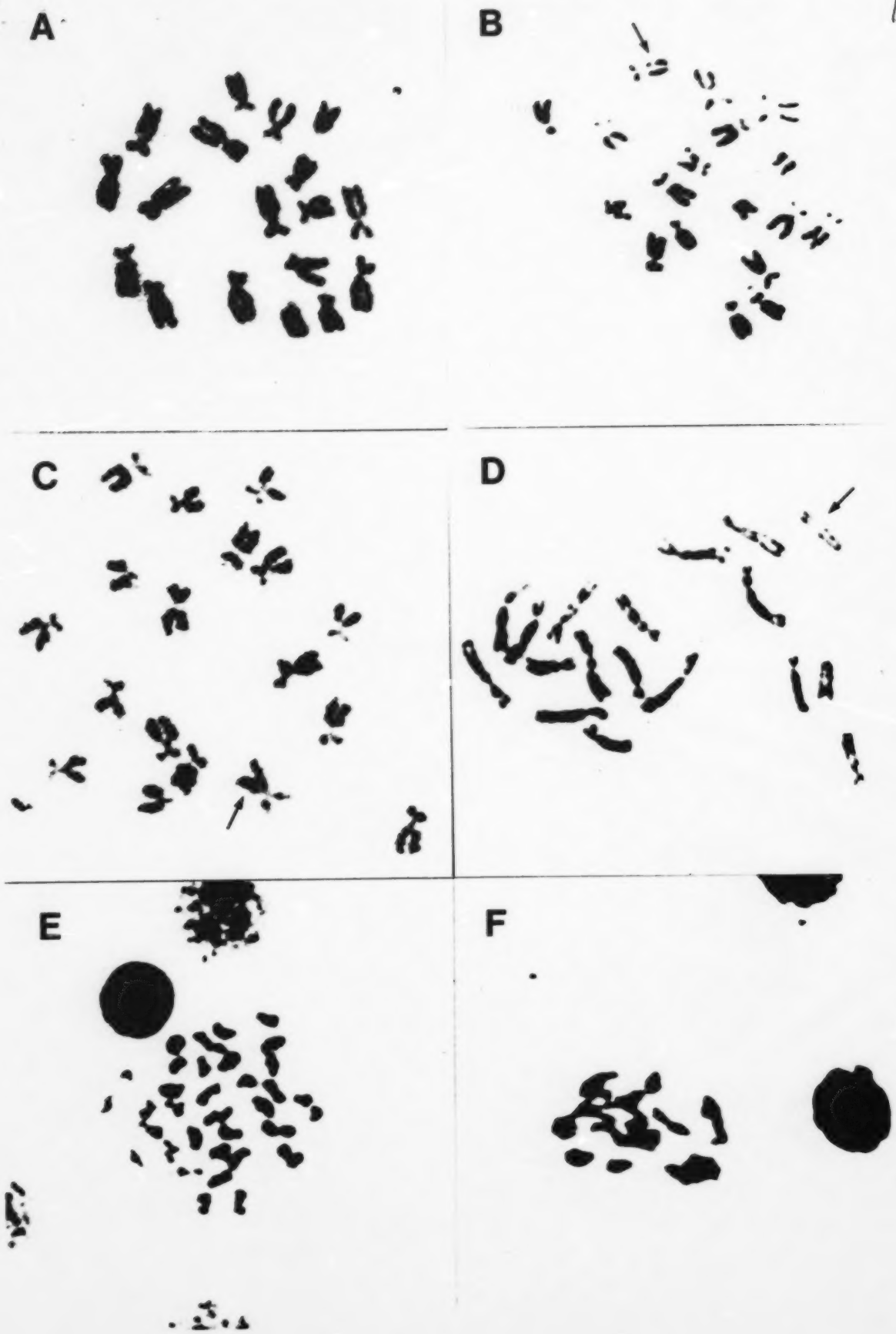
Table 1: Summary of Data
Radiation Effects on N. arenaceodentata Chromosome Morphology

Total Dose (Rads)	Exposure Time	Dose Rate Rads/Day	Number of Worms Examined	Size Range of Worms (mm)	Total Number of* Metaphases	Number of Metaphases Scored	Number of Metaphases Abnormal	Percent Abnormal	Confidence** Interval (95%)	Percent of Abnormals with Multiple Chromosomes Damaged
180 ± 10	24 d	7.5	5	9-15	1168	400	53	13.3	10-17	57
225 ± 13	1 min	$324x10^3$	10	8-20	392	94	45	47.8	38-58	62
680 ± 20	3 min	$326x10^3$	10	9-16	561	545*	544	99.8	98-100	99
Control	-		10	10-20	394	119	1	0.8	0-4	0
Control	-		3	8-20	713	358	2	0.5	0-2	0
677 ± 153	48 d	14	10	8-20	495	244	15	6.1	4-10	27

* Difference between total number and number scored due to number of metaphases not spread well enough to score. The spread due to technique.

**Calculated by a comparison of proportions test for binomial distributions (Snedecor and Cochran, 1967).


ABNORMAL METAPHASES (%)
100
80
60
40
20
0
Rapid Dose (min.)
Slow Dose (wks.)
0
100
200
300
400
500
600
700
TOTAL DOSE (RADS)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